Possible Implications of Bovine Spongiform Encephalopathy for the Safety of Bovine Heparin

The FDA has proposed reintroducing bovine-derived heparin to the US market to benefit public health by diversifying the heparin supply chain. Bovine lung heparin was voluntarily withdrawn from the US market about 20 years ago by manufacturers concerned about the theoretical risk that injected heparin might expose users to the infectious agent of bovine spongiform encephalopathy (BSE). BSE was reported in 1996 to have infected humans in the UK and France. The BSE agent caused a previously unrecognized variant Creutzfeldt-Jakob disease (vCJD) in humans, most likely acquired through consumption of contaminated beef products. Since then, 228 clinical cases of vCJD have been reported (as well as a number of people with possible pre-clinical infections) in seven European countries and five countries outside Europe **(6)**. BSE is the only transmissible spongiform encephalopathy (TSE) of animals known be "zoonotic"—transmissible to humans. As such, the danger of accidental transmission to humans must always be considered when evaluating the safety of FDA-regulated products of bovine origin. This brief summary considers the history of iatrogenic transmissible spongiform encephalopathy (TSE, prion disease), addresses the theoretical possibility that medical products with active ingredients/components of bovine origin might, if contaminated with the BSE agent, transmit vCJD, and introduces some general approaches to manage that risk.

Importantly, although more than 375 iatrogenic cases of typical CJD have been described during the past 40 years, such cases have become increasingly rare, and all have been attributed to products of human origin: grafts of human cornea, dura mater allografts, cadaveric pituitary hormones and neurosurgical instruments **(4)**. More recent cases of transmitted vCJD in the UK have been attributed to transfusions of non-leukoreduced red blood cells and a relatively crude human plasma-derived coagulation factor. Notably, to our knowledge, there has never been a documented or even suggestive transmission of a TSE to humans from a medical product of bovine origin. This is true even in the UK where a very large number of cattle were infected with the BSE agent during the 1980s and early 1990s and there was exceptionally high awareness of the potential risk. On the other hand, as early as the 1930s, many sheep were infected with the scrapie agent (a TSE of sheep and goats) that accidentally contaminated a formalin-inactivated veterinary vaccine in the UK **(1)**. Thus, if a BSE agent contaminated medical products intended for parenteral use by humans, the BSE agent would almost certainly infect some people (parenteral routes generally being far more efficient for transmitting TSEs than the oral route).

When a product with a clear benefit has a potential risk to the user—a risk that cannot be substantially reduced—the risk may have to be accepted, generally with disclosure to possible users. When possible, several kinds of precaution may be taken ("mitigations") to reduce risk to the users: (1) restrict use of the product to those for whom it is strictly indicated; (2) limit raw materials to the safest possible (in this case, least likely to contain the BSE agent); and (3) introduce into the manufacturing process, when feasible, steps expected to eliminate or at least reduce the risk (in this case meaning removal of any BSE agent that might have accidentally contaminated crude heparin starting materials). How

might those general principles of risk management apply to reduce the risk that bovine-derived heparin might transmit a BSE infection to users?

Restrict use of bovine heparin. The practice of medicine dictates that treatments with products like heparin should be used only for reasonable indication. The theoretical BSE risk associated with bovine heparin, and its benefits, should be compared with risks and benefits associated with heparins of other origin in deciding which is likely to be safer.

Limit sources of crude bovine heparin to the safest reasonably possible. BSE is thought to have begun to spread in the UK in about 1980, possibly due to changes in the rendering of ruminant offal that increased the likelihood that either sheep scrapie agent (or a previously unrecognized TSE agent of cattle) contaminated rendered protein (meat-and-bone meal [MBM]) used to supplement cattle feed;. In addition, subsequent rendering of BSE-infected cattle would have amplified the contamination of MBM. Increasingly stringent controls on the use of MBM and similar additives in animal feeds in the UK, starting in 1989, was followed about four years later by a peak in BSE cases there, followed by a marked reduction over the following decade. The total number of recognized BSE cases in the UK since 1986 exceeds 184,000, and many other infected animals must have gone unrecognized. Since 2012, the UK has reported only four cases of BSE to the World Organisation for Animal Health (OIE) **(18)**.

Unfortunately, perhaps resulting from substantial UK exports of MBM (as well as live cattle and beef) presumably contaminated with the BSE agent, 25 other countries, including the US, later recognized BSE in native cattle. Ten countries have reported new BSE cases to the OIE after the end of 2011. Because national reporting of BSE cases is voluntary and recognition generally requires a relatively sophisticated and expensive surveillance and testing program, it is possible that BSE might be present in cattle of other countries. Nonetheless, the probable overall prevalence of BSE infection in many developed countries with active BSE surveillance and testing programs, including the US, appears to be very low.

The OIE has an internationally agreed-upon procedure to recognize the BSE status of cattle from countries that request an evaluation and submit supporting documentation. The lowest-risk countries are considered to have “negligible” BSE risk (currently 25 countries, including the US). Countries with a somewhat higher BSE risk are considered to have “controlled” BSE risk (currently 27 countries including Canada and the UK). Countries that have not submitted information to the OIE allowing assignment to negligible-risk or controlled-risk categories are considered to have “undetermined” BSE risk. The USDA announced, in December 2013 **(12)**, that, with some reservation, it accepted the OIE-assigned risk categories for trade, replacing a list of countries to which BSE-related prohibitions by USDA for importing certain bovine products had previously applied.

Therefore, as a first step in reducing BSE risk for bovine heparin, the country of origin for crude bovine should be one acceptable to the USDA for exporting edible bovine products to the US. Additional stricter criteria might also be considered, for example, (1) assurances that the source cattle were traceable from birth to slaughter, (2) were never fed proteins prohibited in ruminant feed by FDA regulations **(14)**, and (3) that animals came from herds with effective BSE surveillance programs, including holding of older sentinel

animals and active postmortem testing of healthy sentinels as well as disabled animals and animals found dead. The feasibility of reliably certifying suitable source cattle would have to be explored before implementation.

A second approach for assuring that crude bovine heparin is unlikely to be contaminated with the BSE agent would be to source it only from tissues unlikely to contain infectivity, even when collected from carcasses of animals incubating BSE. An expert working group of the World Health Organization (WHO) has published (2006 and last amended in 2010 but still reasonably up-to-date) useful tabular summaries of published reports on both TSE infectivity and TSE-associated abnormal prion protein (PrP^{TSE}) in various tissues of humans with CJD and vCJD and animals with naturally occurring TSEs, including BSE in cattle **(16, 17)**. Lungs and blood from cattle with BSE have not been demonstrated to contain either PrP^{TSE} or infectivity (though both blood and lungs of experimentally infected hamsters have contained scrapie agent **(3)**); the proximal ileum of BSE cattle contained both PrP^{TSE} and infectivity, and recent studies detected small amounts of infectivity wider spread throughout the intestines of animals dosed with the BSE agent orally as calves **(2)**. The intestinal tract (especially its lymphoid and neural tissues) may become infected soon after oral exposure to the BSE agent early in life. Other tissues appear to become infected only later, so the risk for BSE-agent contamination of bovine lung might be lower in younger animals—consistent with USDA regulatory policies for removal of certain tissues at slaughter only for animals older than 30 months. A caveat: studies of the distribution of the BSE agent in various tissues of infected animals during the incubation period and overt illness have been few, usually involved relatively small numbers of animals and their fluids and tissues, and, by necessity, were able to assay only small total volumes. Those problems increase uncertainty regarding the negative predictive value of failures to detect the BSE agent in samples. However, restricting bovine source material for crude heparin to tissues unlikely to contain detectable amounts of infectious agent seems prudent and might be considered.

One extremely important additional step to reduce the risk for contaminating crude bovine-derived heparin with the BSE agent would be to ensure that slaughtering procedures avoid cross-contamination of low-risk tissues with high-risk tissues—mainly brain and spinal cord. Procedures intended to do that, including careful removal of the head and spinal cord from carcasses, have already been implemented by USDA in the past for carcasses of older animals. (In January 2007, a Proposed FDA Rule, not finalized, also addressed these and other issues related to implications of BSE for the safety of FDA-regulated biologics, drugs, medical devices and drugs for animals **(15)**.)

While testing of source tissues for the presence of PrP^{TSE} seems an attractive option at first glance, existing research and commercial assays for detecting PrP^{TSE} have not yet been adequately validated for that purpose. Some intriguing PrP^{TSE} tests in development are claimed to have sufficient sensitivity to become useful, when validated, to screen tissues. Testing of brain tissues of source animals for PrP^{TSE} (for which commercial tests are already available) might provide some additional assurance of safety, but negative test results would not guarantee that other tissues are not contaminated with small amounts of infectivity. Bioassays for infectivity have generally been more sensitive than tests detecting PrP^{TSE} but require a long time and so are not practical.

Incorporation into the manufacturing process of steps to remove BSE agent from crude heparin. Several generic steps in the manufacturing process for heparin might have some capacity to remove or inactivate (theoretically better) some BSE agent present in a crude tissue extract: steps that physically remove TSE agents might include membrane filtration, ion-exchange-resin filtration and solvent precipitations—presuming that the BSE agent was concentrated in discarded fractions and not in heparin-enriched fractions. Physical separations removed substantial amounts of infectivity (>4 log10) when model TSE agents, in pilot studies, were spiked into human plasma, still yielding active therapeutic proteins.

Certain chemical treatments, like additions of concentrated NaOH and NaOCl, have inactivated large amounts of TSE infectivity—to the limit of detection when combined with steam autoclaving **(11)**. Treatment with base at elevated temperature might be a feasible step to inactivate substantial amounts of BSE agent potentially contaminating crude heparin. Other chemical treatments were found effective in eliminating TSE agents from contaminated surfaces **(5, 9, 10)**, and some of those treatments might conceivably be incorporated into the manufacture of heparin. Special attention should be paid to the fact that elimination of TSE agents from contaminated materials has been "context-dependent," that is greatly influenced by the milieu in which the agent is found (state of hydration, aggregation, "organic load" and other properties of the matrix) as well as by probable differences in intrinsic properties of the agents themselves **(8, 11)**. FDA plans to conduct a pilot "process validation" study (based on general concepts developed to estimate the clearance of viruses **(7, 13)**) to evaluate the effects of various typical promising steps in heparin manufacture on spiked infectious TSE agent—preferably BSE agent in tissue suspensions of bovine origin if available.

Conclusions. The safety of FDA-regulated medical products generally requires simultaneous implementation of several different precautions, sometimes called "tiers of safety." In the past, introduction of precautions that reduced the risk of exposure to TSE agents resulted in dramatic decline in incidence of infection. Kuru was eliminated by the single step of ending ritual cannibalism among the Fore People of Papua New Guinea; transmissible mink encephalopathy and, possibly, BSE were effectively controlled by changes in feeding practices. Simultaneous implementation of several mitigations would be expected to reduce substantially the risk that bovine heparin might become contaminated with the BSE agent, even if each mitigation had limitations. Prevalence of pre-clinical BSE in cattle in the US and other countries assigned negligible-risk by the OIE is thought to have become extremely low in recent years. Taken together, national regulations prohibiting certain proteins in ruminant feed **(14)** and import prohibitions have reduced both the spread of BSE and the risk for reintroducing BSE into negligible-risk countries from higher-risk countries. Careful sourcing of animals and selection tissues, perhaps augmented by incorporation into the heparin extraction scheme of validated steps that remove substantial if not all infectivity, would provide an additional tier of safety for bovine heparin.

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